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EDGEWOOD ARSENAL
TECHNICAL REPORT

13731 A CLINICOPATHOLOGIC STUDY OF THE EFFECTS
OF RIOT CONTROL AGENTS ON MONKEYS
8089 Y. LOW CONCENTRATIONS OF DIPHENYLAMINO-
CHLOROARSINE (DM) OR o-CHLOROBENZYLIDENE
MALONONITRILE (CS) FOR EXTENDED PERIODS

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EDGEWOOD ARSENAL TECHNICAL REPORT

EATR 4072

A CLINICOPATHOLOGIC STUDY OF THE EFFECTS OF
RIOT CONTROL AGENTS ON MONKEYS
V. LOW CONCENTRATIONS OF DIPHENYLAMINOCHLOROARSINE (DM)
OR o-CHLOROBENZYLIDENE MALONONITRILE (CS) FOR
EXTENDED PERIODS

by

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January 1967

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Medical Research Laboratory
Research Laboratories
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FOREWORD

The work described in this report was authorized under Task IC522301A07908, Incapacitating and Riot Control Agents (U). This work was started in December 1965 and completed in June 1966.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences — National Research Council.

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DIGEST

This is a report of the order, severity, and resolution of pathologic changes in Macaca mulatta monkeys exposed to low concentrations of DM or CS for extended periods.

The following observations were made:

1. Exposure of the monkeys to low concentrations of CS for relatively long periods (Ct, 265 to 9120 mg min/cu m; t, 5 to 30 min) produced few clinical signs, no systemic manifestations of toxicity, and no pathologic changes.

2. Exposure of the monkeys to low concentrations of DM for relatively long periods (Ct, 198 to 13,200; t, 2 to 60) produced clinical signs and pathologic changes in a significant number of the animals. The clinical signs were primarily nausea and vomiting, and the pathologic changes were bronchorrhea, congestion, edema, and pulmonary inflammation.

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A CLINICOPATHOLOGIC STUDY OF THE EFFECTS OF
RIOT CONTROL AGENTS ON MONKEYS
V. LOW CONCENTRATIONS OF DIPHENYLAMINOCHLOROARSINE (DM)
OR o-CHLOROBENZYLIDENE MALONONITRILE (CS)
FOR EXTENDED PERIODS

I. INTRODUCTION.

Previous reports in this series* deal with concentrations of riot control agents that are significantly greater than those expected in open-air situations. One disadvantage to using high concentrations is that many animals, especially primates, will stop breathing for some time when in a noxious atmosphere. Wide variations among the responses of individual animals can therefore be expected, depending on the extent of breathholding. Another disadvantage is that high concentrations evoke a maximal respiratory response; that is, marked bronchospasm, coughing, gagging, edema, and bronchorrhea. The responses tend to dilute or wash out the agent.

An additional factor clearly indicated a need for further evaluation. Many of the monkeys used as experimental animals have preexisting emphysema and atelectasis, which also occur in cigarette smokers. The high doses of agent used in the previous studies produced such severe pulmonary lesions that it was not possible to ascertain whether the agent was accentuating the preexisting lesions or whether it was producing superimposed lesions. An experiment using lower doses, which would not be expected to obscure the effect on preexisting lesions, was therefore designed.

* Striker, G. E., Streett, C. S., Ford, D. F., Herman, L. H., and Helland, D. R. EATR 4068. A Clinicopathologic Study of the Effects of Riot Control Agents on Monkeys. I. Diphenylaminochloroarsine-Chloroacetophenone (DM-CN) Munition. UNCLASSIFIED Report. (In press.)

Striker, G. E., Streett, C. S., Ford, D. F., Herman, L. H., and Helland, D. R. EATR 4069. A Clinicopathologic Study of the Effects of Riot Control Agents on Monkeys. II. Chloroacetophenone (CN) Grenade. UNCLASSIFIED Report. (In press.)

Striker, G. E., Streett, C. S., Ford, D. F., Herman, L. H., and Helland, D. R. EATR 4070. A Clinicopathologic Study of the Effects of Riot Control Agents on Monkeys. III. Diphenylaminochloroarsine (DM) Grenade. UNCLASSIFIED Report. (In press.)

Striker, G. E., Streett, C. S., Ford, D. F., Herman, L. H., and Helland, D. R. EATR 4071. A Clinicopathologic Study of the Effects of Riot Control Agents on Monkeys. IV. o-Chlorobenzylidene Malononitrile (CS) Grenade. UNCLASSIFIED Report. (In press.)

This is a report of the order, severity, and resolution of pathologic changes in monkeys exposed to low concentrations of DM or CS for extended periods.

II. MATERIALS AND METHODS.

A. Munitions.

DM was dispersed from an M6A1 grenade and CS was dispersed from an M7A3 grenade. Both munitions were obtained from standard US Army supply sources.

B. Animals.

Male and female Macaca mulatta monkeys, weighing 3 to 4 kg, were conditioned for 1 mo and tuberculin-tested before being considered for experimental use. Control and experimental monkeys were picked at random from the same group. They were treated identically except that the control monkeys were not put into the exposure chamber.

C. Exposure.

Thirty monkeys, in groups of 5, and 35 monkeys, in groups of 5, were exposed to CS and DM, respectively. The 20,000-l exposure chamber contained a cloud generated by the munition. The cloud was sampled at various times, and the concentration of agent was measured. A summary of the Ct's* to which the monkeys were exposed is shown in table I.

D. Serial Sacrifice.

One monkey from each group of five exposed to agent was sacrificed by the intravenous administration of sodium pentobarbital 12 hr, 24 hr, 72 hr, 1 wk, or 30 days after exposure. The 14 controls for the DM study were sacrificed similarly at 1 wk and 30 days, and the nine controls for the CS study were sacrificed at 12 hr, 1 wk, and 30 days. All monkeys were necropsied immediately after sacrifice.

* Ct is concentration in milligrams per cubic meter times duration of exposure in minutes. The units for Ct (mg min/cu m) will not be repeated in the text of this report.

Table I. Ct's of DM or CS to Which Monkeys Were Exposed†

Concentration (C)		Time (t)	Ct
Goal	Actual		
mg/cu m		min	mg min/cu m
A. <u>DM</u>			
300	291	2	582
	291	10	2910
	272	20	5440
	330	40	13,200
100	99	2	198
	108	12	1296
	77	60	4620
B. <u>CS</u>			
50	53	5	265
	55	10	550
	50	30	1500
300	305	5	1525
	307	10	3070
	304	30	9120

† Five monkeys were exposed to each Ct.

E. Radiographs.

Chest radiographs were taken with a Fexitron No. 846 portable unit* just before the animals were exposed and 2 hr, 6 hr, 12 hr, 24 hr, 72 hr, 1 wk, and 30 days after exposure or more frequently if clinically indicated. Each monkey was first given Sernyl intramuscularly to facilitate handling and to reduce motion artifacts. The monkeys were always positioned a fixed distance from the X-ray source.

F. Clinical Evaluation.

All animals were examined by one of the authors before exposure. For the first 72 hr after exposure, observations were made frequently and then as clinically indicated.

* Field Emission Corp.

III. RESULTS.

No lesions attributable to agent exposure were detected radiographically.

A. Control Animals.

Lesions seen in the control animals consisted mainly of microscopic foci of atelectasis and emphysema with associated fibrosis. These were assumed to be related to a rather heavy infestation by the lung mite, Pneumonyssus simicola. The same types of lesions were also seen routinely in the experimental animals.

B. CS Exposures.

Clinical signs in all animals exposed to CS (Ct, 265 to 9120; t, 5 to 30) were blinking, mild coughing, mild nasal discharges, and some rubbing of the eyes. No evidence of excess nasal-pharyngeal exudate was noted.

The only findings on necropsy were lung-mite lesions that were comparable to those seen in the controls.

C. DM Exposures.

The clinical signs seen in the monkeys exposed to DM are listed in table II. Because lesions were confined to the pulmonary system, necropsy descriptions are limited to that system. The necropsy findings are listed in table III. No lesions attributable to agent exposure were detected radiographically. Lung weights (figure 1) generally correlated with the presence of pulmonary edema, congestion, and pneumonia.

1. Concentration of About 300 mg/cu m. Varying Exposure Times.

a. Ct, 582.

The only clinical signs noted were modest hyperactivity during exposure and blinking for 10 min immediately after exposure.

The only lesion seen in the monkeys sacrificed at 12, 24, and 72 hr was slight pulmonary congestion. One week after exposure and, more prominently, 30 days after exposure, there were increased congestion and edema (figure 2).

Table II. Clinical Signs Observed in Monkeys Exposed to DM

Ct	Clinical signs
mg min/cu m	
A. <u>C of About 300 mg/cu m; Varying t's</u>	
582	Modest hyperactivity during exposure and blinking
2910	Modest hyperactivity during exposure and blinking
5440	Modest hyperactivity during exposure, blinking, depression, and vomiting
13,200	Conjunctival congestion, depression, oral and nasal discharges, vomiting; and dyspnea
B. <u>C of About 100 mg/cu m; Varying t's</u>	
198	Mild blinking
1296	Blinking
4620	Tearing, blinking, depression, rapid respiration, gasping, and trace oral and nasal discharges

b. Ct, 2910.

Clinical signs in this group were identical to those experienced by the group exposed to a Ct of 582.

Focal pulmonary edema and bronchorrhea were seen in the monkey sacrificed at 12 hr. The edema had cleared by 24 hr, but bronchorrhea and mild bronchitis were still present at 30 days.

c. Ct, 5440.

Immediate postexposure signs were similar to those seen in the two groups exposed to lower Ct's; however, the monkeys were considerably less active. Almost all animals were vomiting by 40 to 60 min after exposure. All signs had disappeared by the second hour, and no further clinical abnormalities were noted.

Table III. Summary of Lesions Found in Monkeys Exposed to DMS

Lesions	Ct, 582						Ct, 2910						Ct, 5440						Ct, 13,200						Ct, 198						Ct, 1296						Ct, 4620										
	12 hr	24 hr	72 hr	1 wk	30 days		12 hr	24 hr	72 hr	1 wk	30 days		12 hr	24 hr	72 hr	1 wk	30 days		12 hr	24 hr	72 hr	1 wk	30 days		12 hr	24 hr	72 hr	1 wk	30 days		12 hr	24 hr	72 hr	1 wk	30 days		12 hr	24 hr	72 hr	1 wk	30 days						
Pulmonary edema				+	+						+	+																																			
Pulmonary congestion	+	+	+	+	+						+	+																																			
Pulmonary hemorrhage											+	+																																			
Emphysema											+	+																																			
Aspiration																																															
Interstitial pneumonia																																															
Bronchopneumonia																																															
Bronchorrhea																																															
Bronchitis																																															
Tracheitis																																															
Mucous plug in bronchus																																															
No lesions																																															

* One monkey sacrificed at each time period.

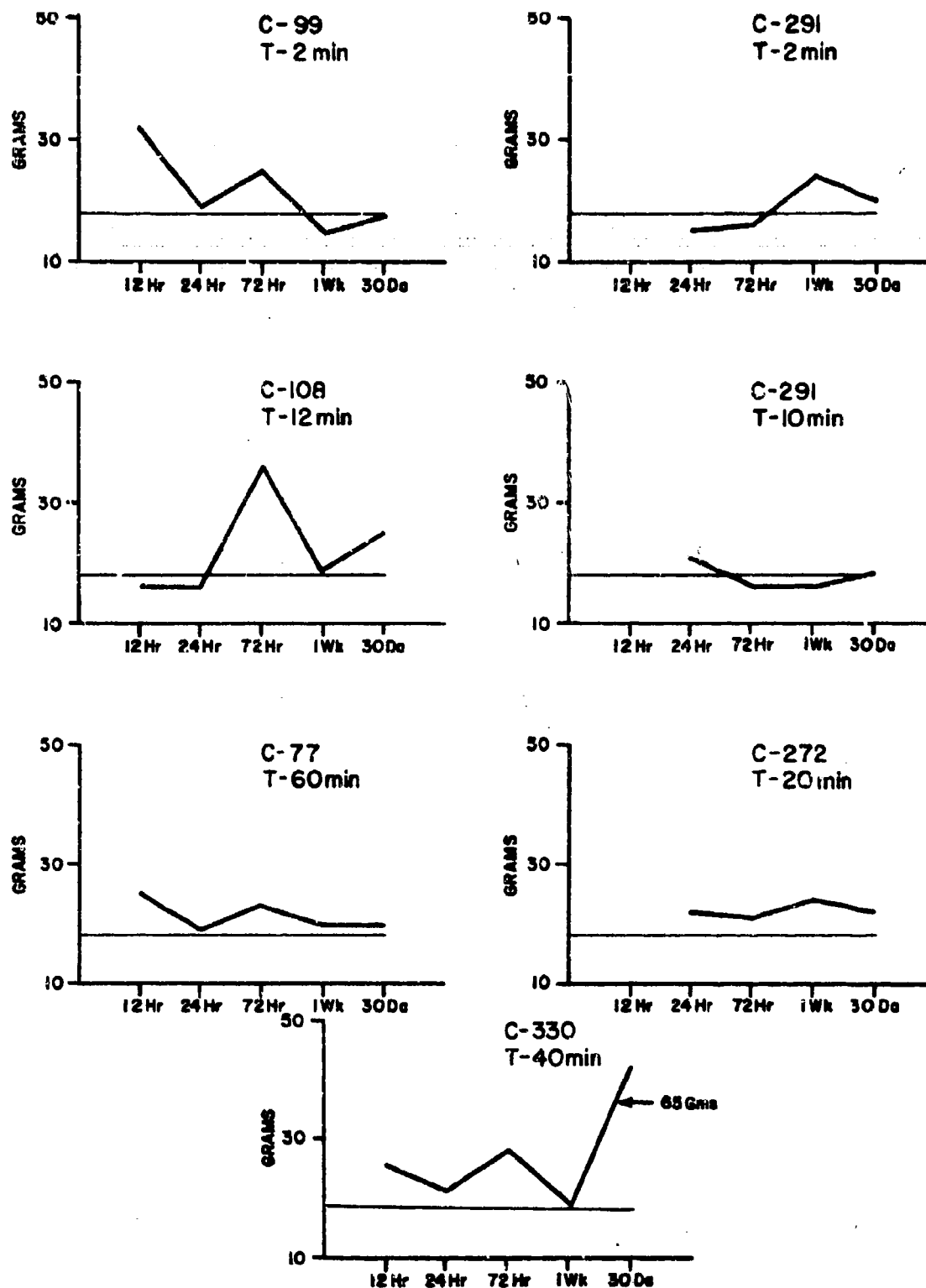


Figure 1. Lung Weights of Monkeys Exposed to DM



Figure 2. Lungs of a Monkey Exposed to DM (Ct, 582) 30 Days Earlier
(Lungs are heavy, severely congested focally, and edematous)



Figure 3. Lungs of a Monkey Exposed to DM (Ct, 5440) 1 wk Earlier
(Note emphysema, bronchorrhea, congestion, hemorrhage, and edema)

The monkey sacrificed at 12 hr had marked bronchorrhea; this had cleared in the animal sacrificed at 24 hr. No lesions were seen in the monkey sacrificed at 72 hr. Pulmonary edema, congestion, hemorrhage, emphysema, and bronchorrhea (figure 3) were seen in the monkey sacrificed 1 wk after exposure. By 30 days, bronchorrhea, bronchitis, pulmonary congestion, hemorrhage, and edema were prominent, and some emphysema was seen.

d. Ct, 13,200.

Conjunctival congestion, marked depression,* and oral and nasal discharges were noted when the monkeys were removed from the chamber. Within 1 hr, most animals were vomiting material that appeared to be swallowed discharges. These signs were still evident at 12 hr, but all except depression had disappeared by 24 hr. The depression persisted to 48 hr and was absent at 72 hr. Dyspnea was seen in all animals at 12 hr, had cleared in some by 24 hr, and had disappeared by 72 hr.

Superficial tracheitis, bronchorrhea, pulmonary edema, congestion, and emphysema appeared at 24 hr and were present in all animals sacrificed at later time periods. The animal sacrificed after 1 wk also had bronchopneumonia and interstitial pneumonia (figure 4); this was replaced by massive pulmonary edema, congestion, and hemorrhage at 30 days (figure 5).

2. Concentration of About 100 mg/cu m;
Varying Exposure Times.

a. Ct, 198.

In this group of monkeys, clinical signs were restricted to mild blinking.

The animal sacrificed 12 hr after exposure had a large mucous plug in a main-stem bronchus, and its lungs were edematous and congested. Bronchorrhea was also quite apparent. The congestion and edema had cleared by 72 hr, and bronchorrhea had resolved by 1 wk. The animal sacrificed at 30 days had evidence of aspiration as well as bronchorrhea, pulmonary congestion, and focal hemorrhages.

* Decreased activity and response to external stimuli.

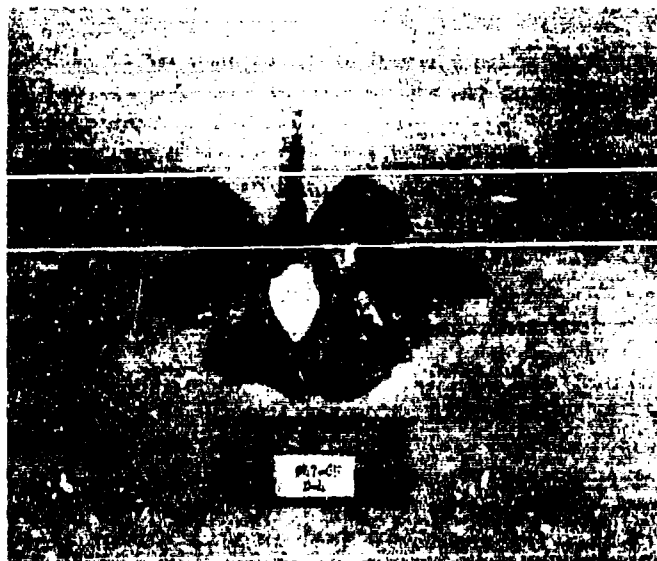


Figure 4. Lungs of a Monkey Exposed to DM (Ct, 13,200) 1 wk Earlier
(Bronchopneumonia is present)

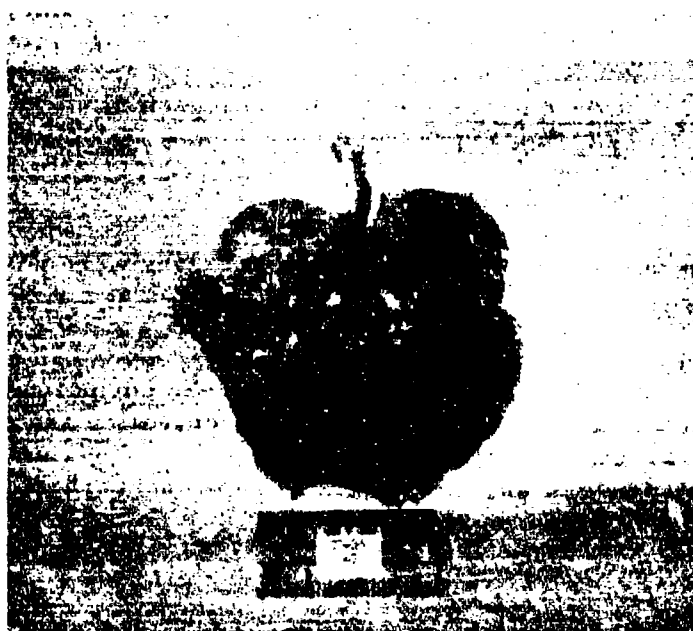


Figure 5. Lungs of a Monkey Exposed to DM (Ct, 13,200)
30 Days Earlier

(Massive congestion, hemorrhage, and edema
are apparent)

b. Ct, 1296.

The only clinical sign noted in this group was blinking.

The monkeys sacrificed 12 and 24 hr after exposure had moderate pulmonary congestion and focal areas of edema. These processes were accentuated in the monkey sacrificed at 72 hr. At 1 wk, focal interstitial pneumonia was seen, and at 30 days, congestion and edema were again prominent.

c. Ct, 4620.

Immediately after exposure, this group of monkeys was tearing and blinking. Depression and a rapid respiratory rate were also noted. Only a trace of oral or nasal discharge was evident. Four hours after exposure, these animals were significantly more depressed and were gasping. Auscultation 10 hr after exposure, when respirations were slow and labored, revealed a marked accumulation of edema fluid. Considerable improvement was noted at 24 hr, and by 48 hr, all animals had completely recovered clinically.

The necropsy findings parallel the clinical observations. Pulmonary edema, congestion, and bronchorrhea were still present 72 hr after exposure but had disappeared in the animal sacrificed at 1 wk. No significant lesions were present in the monkey sacrificed 30 days after exposure.

IV. DISCUSSION.

After the lowest dose of DM given in the earlier study (Ct, 2565; t, 3),* only one of the 10 monkeys had any lesions; they were superficial tracheitis, edema of the tracheal and bronchial mucosa, and a beginning bronchorrhea. In contrast, the findings in the present study of DM were very vivid. However, the value for concentration present in a chamber is an average of the actual concentrations present at various intervals during the exposure period. When Ct is high and t is low, the peak concentration is held for only a short time, and an animal holding its breath will receive a lower dose than the calculated Ct. A more stable concentration and a more reliable delivered dose would be expected when lower concentrations and longer exposure times are used. This may be particularly significant with a compound such as DM, which has fewer local irritant but more prominent systemic effects than some other riot control agents. Nausea and vomiting, manifestations of systemic effects, occurred in the current study, but did not occur in the earlier study.

* EATR 4070.

Somewhat puzzling, however, was the observation that although early signs after the lower Ct's of DM were relatively minor, a number of the animals developed progressively more-severe pulmonary edema and one developed pneumonia. A hiatus between the early signs and later morbidity was apparent in several groups. These animals were in a state of rest (small cages restricting movement), and the environmental temperature was even. A population subjected to severe stress in the form of vigorous physical activity or extremes of temperature might possibly experience even more-severe manifestations of these reactions to DM.

Because relatively severe lesions were seen at low Ct's of DM, these results should be rechecked. The possibility of an intercurrent viral or bacterial disease seems unlikely because control animals were not affected similarly. Exposure to DM apparently lowered the resistance of the pulmonary parenchyma to subsequent injury. The pulmonary edema and later polymorphonuclear leukocyte response are manifestations of early pneumonia, and we do not have enough information to tell whether the pulmonary edema is a response to the agent or a reflection of secondary bacterial infection. The former is more likely in view of the uniformity of this response. It is also not possible to say that preexisting lesions were accentuated by the agent.

The concentrations used in this study are at the upper limit of what might be expected in an open-air situation, and the exposure periods certainly are much longer than one would expect in such a situation. These results, however, emphasize that use of this agent is not without hazard.

CS, in contrast to DM, appears to have no cumulative or systemic effects when delivered at relatively low concentrations for long periods. No lesions were seen. In the earlier study,* two Ct's were similar to those used in the present study (Ct, 3070; t, 10 and Ct, 9120; t, 30), but they were obtained by exposing the monkeys to higher concentrations for shorter times (Ct, 2700; t, 3 and Ct, 8500; t, 5). Many pulmonary lesions were seen in these animals.

It would appear from the present study that, at comparable concentrations and time periods, CS is considerably less toxic than DM, both initially and for at least 30 days. The fact that CS also has immediate irritant effects in man makes it the agent of choice for many riot control situations.

* EATR 4071.

V. CONCLUSIONS.

The following observations were made:

1. Exposure of the monkeys to low concentrations of CS for relatively long periods (Ct, 265 to 9120 mg min/cu m; t, 5 to 30 min) produced few clinical signs, no systemic manifestations of toxicity, and no pathologic changes.

2. Exposure of the monkeys to low concentrations of DM for relatively long periods (Ct, 198 to 13,200; t, 2 to 60) produced clinical signs and pathologic changes in a significant number of the animals. The clinical signs were primarily nausea and vomiting, and the pathologic changes were bronchorrhea, congestion, edema, and pulmonary inflammation.

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13. ABSTRACT (U) This is a report of the order, severity, and resolution of pathologic changes in <u>Macaca mulatta</u> monkeys exposed to low concentrations of DM or CS for extended periods. The following observations were made: (1) Exposure of the monkeys to low concentrations of CS for relatively long periods (Ct, 265 to 9120 mg min/cu m; t, 5 to 30 min) produced few clinical signs, no systemic manifestations of toxicity, and no pathologic changes. (2) Exposure of the monkeys to low concentrations of DM for relatively long periods (Ct, 198 to 13,200; t, 2 to 60) produced clinical signs and pathologic changes in a significant number of the animals. The clinical signs were primarily nausea and vomiting, and the pathologic changes were bronchorrhea, congestion, edema, and pulmonary inflammation.			
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